IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application Serial No:	09/593,914
Date Filed:	June 14, 2000
Application Title:	Probes, Probe Sets, Methods And Kits Pertaining To The Detection, Identification And/Or Enumeration Of Yeast; Particularly In Wine
Applicants:	Hyldig-Nielsen et al.
Group Art Unit:	1655
Examiner:	C. Myers
Action Date:	July 18, 2001
Action Type:	First Office Action On Merits - NON-FINAL
Certified Mail No.:	7099 3400 0007 5728 5682

Certificate of Mailing Pursuant to:
37 C.F.R. § 1.8

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to: Commissioner for Patents, Washington, DC 20231 on this 17th day of January, 2002.

Brian D. Gildea
Reg. No. 39,995

DECLARATION UNDER 37 C.F.R. § 1.131
TO OVERCOME A 35 U.S.C. §102(a) REJECTION

Commissioner for Patents
Washington, DC 20231

Dear Sir or Madam:

We, Jens J. Hyldig-Nielsen, of 1460 Highland Street, Hollison, MA, Heather P. O'Keefe of 248 Marrett Road, Lexington, MA and Henrik Stender of 137 Fox Road, #402, Waltham, MA do hereby declare and state that:

BP9901-US

1. We are the inventors of the above captioned patent application;

2. We are also identified as co-authors of the publication: Stender, H. et al., A new molecular method for simultaneous identification and enumeration of Brettanomyces in wine. Abstracts of the General Meeting Of The American Society For Microbiology, 99: 516 (1999) (copy attached as Exhibit A).

3. The invention as described and claimed in the present application was completed, based upon our work performed in the United States of America, prior to the publication date of said abstract;

4. The work described in said published abstract was derived from knowledge of our work based upon the participation of all of the identified authors in a program designed to produce a commercial product for the determination of Brettanomyces in wine;

5. The identified authors, other than ourselves, are not inventors of the subject matter claimed in the present application, but were identified as authors of the published abstract in conformance with professional standards used in determining authorship for publications.

We further declare that all statements made in this Declaration are true and that all statements made on information and belief are believed to be true. Moreover, these statements were made with the knowledge that willful false statements and the like made by me are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Jens J. Hældig-Nielsen — Date 01/17/02

Heather P. O'Keefe — Date 1/17/02

Henrik Stender — Date 1/17/02

EXHIBIT A

P.D. 31-05-1979
P. 5160 (1)
XP-000952544

P-26. A New Molecular Method for Simultaneous Identification and Enumeration of *Brettanomyces* in Wine

H. STENDER[1], H. PERRY-O'KEEFE[1], J.J. HYLDIG-NIELSEN[1], A. BROOMER[1], M. SARACINO[1], C. KURTZMAN[2], B. YOUNG[3], J.M. COULL[1], [1]Boston Probes, Inc., Bedford, MA; [2]Natl. Ctr. for Agric. Utilization Res., Peoria, IL; [3]Millipore, Bedford, MA

Brettanomyces (ascosporic state of *Dekkera*) are well recognized as spoilage yeasts in wine causing 'mousiness', an undesirable odor and taste. Current methods for identification and enumeration take 1-2 weeks and rely on semi-selective culture medium followed by final identification from morphology and biochemical testing. A new *in situ* hybridization method using peptide nucleic acid (PNA) probes for simultaneous identification and enumeration of *Brettanomyces* within 2 days has been developed. The wine sample is filtered to isolate and separate individual microorganisms onto a membrane which is placed on a culture medium for up to 44 hours prior to testing. Microscopic colonies of *Brettanomyces* are detected on the membrane by hybridization with peroxidase-labeled PNA probes targeting *Brettanomyces* 26S rRNA. Excess probe is removed by washing and hybridized probe is visualized by a chemiluminescent reaction. Each *Brettanomyces* micro-colony is observed as a small dot providing simultaneous identification and enumeration. 100% sensitivity and 100% specificity of the probes have been determined using reference strains and wine isolates of *Brettanomyces* as well as other yeast species potentially found in wine. Results obtained using the method to detect *Brettanomyces* in wine samples will be presented.

BP9901-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application Serial No:	**09/593,914**	**Confirmation No: 8319**
Date Filed:	June 14, 2000	
Application Title:	Probes, Probe Sets, Methods And Kits Pertaining To The Detection, Identification And/Or Enumeration Of Yeast; Particularly In Wine	
Applicants:	Hyldig-Nielsen et al.	
Group Art Unit:	1634	
Examiner:	C. Myers	
Action Date:	February 25, 2003	
Action Type:	Third Office Action On Merits - FINAL	
Certified Mail No.:	7003 0500 0000 1731 7079	

Certificate of Mailing Pursuant to:
37 C.F.R. § 1.8

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to: Mail Stop: AF, Commissioner For Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on this 22nd day of August, 2003.

Brian D. Gildea
Reg. No. 39,995

DECLARATION OF DR. HENRIK STENDER
UNDER 37 C.F.R. § 1.132

Commissioner for Patents
Dear Sir or Madam:

I, Dr. Henrik Stender of Fasanhaven 5, DK-2820 Gentofte, Denmark do hereby declare and state that:

1. I am presently employed as Vice President of Research & Development of AdvanDx, Inc. and have been in this position since August 2002;

2. I was formerly employed at Boston Probes & Applied Biosystems from May, 1998 until August, 2002 in various positions, including Director of Microbiology;

3. Before that I was employed at Dako A/S Denmark as Research Scientist from July, 1992 until May, 1998;

4. I received my doctorate from Technical University of Denmark in 1992 in the area of Immunology;

5. I have been employed as a scientist/manager in the field of assay development for a total of 11 years;

6. I am a co-inventor of the above captioned patent application and I have reviewed the claims as currently pending in the application;

7. I have reviewed the Office Action dated February 25, 2003 and the examiners arguments set forth therein;

8. I am a co-inventor of PCT/DK97/00425 (WO98/15648) entitled: "Novel Probes For The Detection Of Mycobacteria", referred to by the Examiner in the above captioned application as Stender (1998) and am familiar with its contents;

9. I have reviewed Kosse et al., Systems. Appl. Microbiol. 20: 468-480 (1997);

10. I have reviewed Amann et al., Applied and Environmental Microbiology 58(9): 3007-3011 (1992);

11. Based upon my review of the Amann et al. reference, I believe that: 1) Amann et al. teach that it was well accepted, at the time of their publication, that enzyme-linked (labeled) probes COULD NOT readily penetrate the cell wall of yeast; 2) Amann et al. had no success with getting enzyme-labeled probes into yeast; and 3) Amann et

al. would tend to dissuade one of skill in the art from attempting to use an enzyme-linked probe to analysis a yeast in an in-situ based assay;

12. Kosse et al. would not, in view of Amann et al., tend to motivate the application of enzyme-linked probes to in-situ assays for yeasts because the reference does not address this particular assay format;

13. Stender (1998) would not, in view of Amann et al. tend to motive the application of enzyme-linked probes to in-situ assays for yeasts because the reference does not address yeasts;

14. One of skill in the art at the time of the present invention would not have a reasonable expectation of successfully applying enzyme-linked probes to the determination of yeast in an in-situ based assay because there was inadequate teaching available as to how to permeablize the cell wall of the yeast to these large molecules.

I further declare that all statements made in this Declaration are true and that all statements made on information and belief are believed to be true. Moreover, these statements were made with the knowledge that willful false statements and the like made by me are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Henrik Stender

8/21 - 2003
Date